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ABSTRACT
Conventional wastewater treatment have proved to be highly efficient on domestic effluents. On the contrary, other anthropogenic effluents such as landfill leachate require complex and specific treatments. In this study the efficiency of attached fungal biomass in landfill leachate treatment has been evaluated according to different experimental conditions. Landfill leachate was treated using the white rot fungus *Bjerkandera adusta* MUT 2295 on biofilm, through batch and continuous tests. Enzymatic activity was assayed in bacth tests in a) 1:1 diluted leachate, b) 1:2 diluted leachate, c) pretreated leachate with aerobic granular sludge and raw leachate with two different co-substrates d) glucose 1 g/L and e) malt extract 1 g/L. Continuous tests on diluted leachate (1:1) are currently ongoing under non sterile conditions. The treatment has been operative for 60 days resulting in a maximum chemical oxygen demand (COD) removal of 40% without additional carbon source.

Keywords: bioreactors, fungal biofilms, landfill leachate.

INTRODUCTION
One of the main problems when treating wastewater is the removal of recalcitrant compounds (González, 2015) since conventional biological treatments, exemplified in activated sludge or trickling filter plants, are not effective in dealing with industrial or highly polluted wastewaters as landfill leachate (Lopez, 2002).

Landfill leachate is generally characterized by high concentrations of biodegradable and non biodegradable compounds, including organic matter, phenols, ammonium nitrogen, phosphate, heavy metals, and sulphide (Kamaruddin, 2014). Among leachate treatment strategies, one of the most commonly applied is the combination of landfill leachate with domestic sewage (Neczaj, 2007), although leachate high organic content negatively affects its efficiency increasing effluent concentrations (Renou, 2008). Nowadays, several drawbacks related to leachate treatment, such as high costs and complexity, are still unresolved and the development of more sustainable technologies represents a major challenge in the field (Kamaruddin, 2014).

For that purpose, treatment with white rot fungi appear as a valuable alternative due to their capability of oxidising complex compounds such as lignin (Lopez, 2002; Kirk and Farrell, 1987). This feature is based on the extracellular secretion of highly oxidative oxidases and peroxidases. The most effective enzymes are lignin peroxidase (LiP), manganese peroxidase (MnP) (Glenn, 1986; Tien, 1988) and laccases (LaC) (Kalčíková, 2014; Wesenberg, 2003).

Because of extracellular enzymes versatility, fungal treatment, compared to bacterial one, provides easier degradation of complex pollutants and higher COD reduction rates towards different problematic wastewaters (Ellouze, 2008).

Besides remarkable results have been attained through batch-scale fungal experiments on leachate, continuous or full-scale treatments are still poorly applied. The main disadvantages are the requirements of nutrients, low optimal pH, production of mycelia and an additional step to remove ammonium nitrogen (Kalčíková, 2014) together with the difficulties in maintaining long-term operation under non-sterile conditions in fungal bioreactors (Gullotto, 2014).

In the present study the capability of *Bjerkandera adusta* MUT 2295 to treat raw leachate was investigated through batch and continuous tests.

MATERIAL AND METHODS

The fungal strain *B. adusta* MUT 2295 was obtained from the *Mycotheca Universitatis Taurinensis* collection (MUT, University of Turin, Italy).

Landfill leachate was collected from Brady Road Resource Management Facility (Brady Road Landfill, Winnipeg, Canada). Chemical and ecotoxicological features (phytotoxicity expressed as inhibition of *Vicia faba* minor root elongation) of raw leachate (RL) and GRL were measured.

Batch tests

B. adusta MUT 2295 was immobilized on 2 cm^3 Polyurethane Foam cubes (PUF). The ability of the fungus to produce MnP was tested for 10 days in a) 1:1 diluted leachate (L50%), b) 1:2 diluted leachate (L33%), c) leachate pretreated with aerobic granular sludge for the removal of ammonium nitrogen (GRL), d) RL with glucose 1g/L and e) RL with malt extract 1g/L. In addition, in the trials d and e, biomass before and after the treatment and color removal were measured. Unseeded controls were performed for all the trials.

Continuous experiments

Continuous experiments on L50% are currently ongoing using 5 L reactors inoculated with *B. adusta* MUT 2295 immobilized on PUF. The Hydraulic Retention Time (HRT) is 72 h. COD and Soluble COD (sCOD) reductions are used as main efficiency parameters. Total Organic Carbon (TOC), pH and enzymatic activities are also evaluated. Glucose was added as co-substrate during the first 15 days of treatment: 1 g/L during the first week and 0.5 g/L during the second one.

RESULTS

Table 1. Raw leachate (RL) and leachate pretreated with aerobic granular sludge (GRL) characteristics. SEM indicates standard error of the mean. * excluding COD/sCOD due to nitrite.

	RL		GRL	
	(mg/L)	**+/- SEM**	**(mg/L)**	**+/- SEM**
COD	1750	150	1352*	102*
sCOD	1566	58	1102*	120*
NH_4^+–N	1150	450	8	3
NO_2^-–N	-	-	464	97
NO_3^-–N	-	-	607	83
Root inhibition (%)	52	-	26	-

MnP activity in diluted leachate showed higher values in the most diluted sample with a maximum of 23.6 U/L (Figure 1a). The lowest MnP production was found in GRL which was characterized by negligible ammonium nitrogen concentration (Table 1), suggesting that MnP in *B. adusta* was presumably negatively affected by its high nitrite concentration.

Phytotoxicity results indicated a significant difference (t-test, P < 0.01) between root elongation in the three tested leachate compared to the control (deionized water). Adversely to the trend observed for MnP activity, GRL showed lower root elongation inhibition compared to diluted leachate samples.

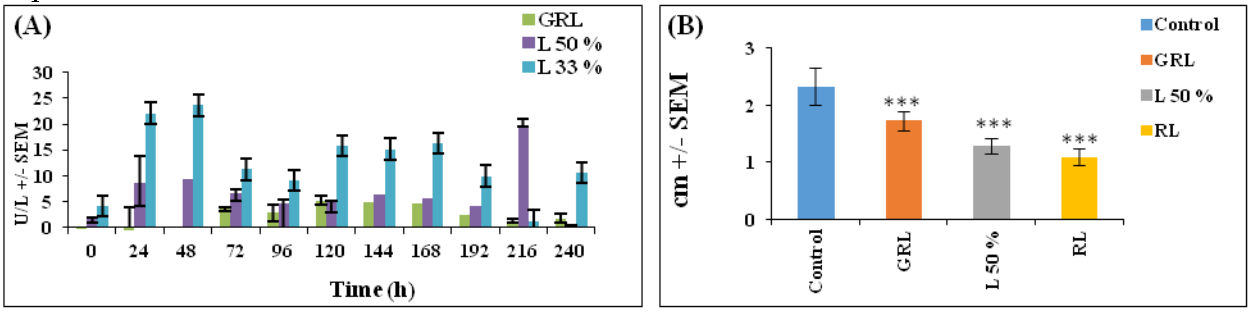

Figure 1. a) MnP activity in L 33%, L 50% and GRL and b) phytotoxicity assayed with *Vicia faba minor.* *** indicates P < 0.01.

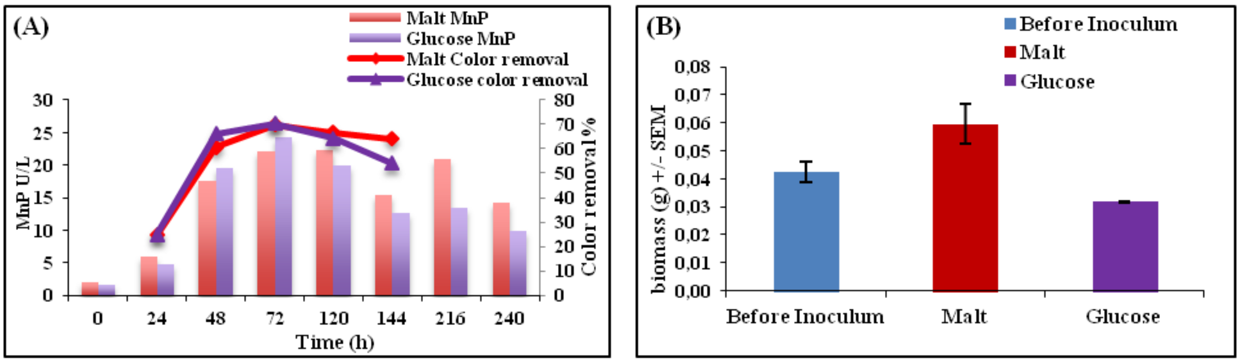

Figure 2. a) MnP activity in raw leachate with malt and glucose and color removal (%) measured during the treatment and b) dry weight of biomass in the PUF before and after treatment .

MnP activity in raw leachate has a maximum value of 24.2 U/L in the trial with glucose after 72 h (Figure 2a) in correspondence to the highest color removal percentage (70%), suggesting the presence of a degradative process in leachate due to fungal treatment.

In the trial with raw leachate and malt, a 40% increase of dry weight of biomass in the PUF, compared to the end of the immobilization phase, was found (Figure 2b). On the contrary, no biomass growth was detected in the trial with glucose.

Up to date, COD removal pattern of the continuous treatment can be divided in two phases according to co-substrate addition (Figure 3). In the first phase (0-17 days), the maximum removal was 54%, which is 5% lower than glucose (1 g/L) theoretical COD (1007 mg/L), suggesting a reduction due to its consumption. In the second phase, COD removal reached a maximum of 40 % in both soluble and total COD after 46 and 51 days respectively, indicating a degradative process related to the treatment. Since aerobic granular sludge (average GRL effluent are shown in table 1) moderately reduced Brady raw leachate COD, the observed removal in L 50% seems particularly promising, indicating that the fungal treatment applied could achieve results unreachable with most common approaches, such as aerobic granular sludge.

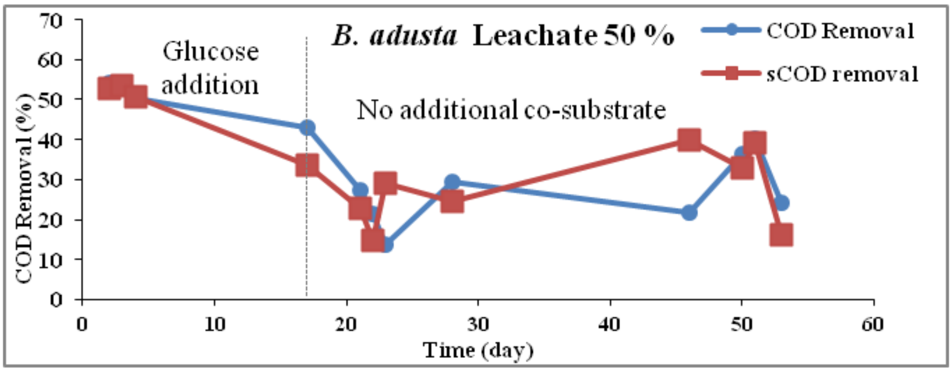

Figure 3. COD and sCOD removal (%) in continuous treatment with *B.adusta* MUT2295.

CONCLUSIONS

The results attained with *B. adusta* MUT 2295, although preliminary, gain a double meaning due to the poor application of fungal biofilm reactors and to the complex nature of landfill leachate. Therefore, further exploitation of this treatment are encouraged in order to optimize its own efficiency.

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